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THE SHAPE OF CONTENTION: ADAPTATION, HISTORY, AND CONTINGENCY IN UNGULATE MANDIBLES

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Mandibles and teeth of ungulates have been extensively studied to discern the functional significance of their design. Grazing ungulates have deeper mandibles, longer coronoid processes, flatter incisor arcades, and more hypsodont molars in comparison to browsers. If the functional significance of both mandible and teeth shapes is well-established, it remains uncertain to what extent mandible shapes are really adapted to grazing, meaning that they evolved either to serve their current biological function or just as a structural requirement to accommodate higher crowned molars. Here, we address this question by studying the contribution of phylogeny, hypsodonty, and body size to mandibular shape variation. The mandible shape appeared to be significantly influenced by hypsodonty but not by body size. Interestingly, hypsodonty-related changes influenced the tooth row in artiodactyls and perissodactyls significantly but in the opposite directions, which is ultimately related to their different digestive strategies. Yet, we obtained a strong phylogenetic effect in perissodactyls, suggesting that their mandible shape should be strongly inherited. The strength of this effect was not significant within artiodactyls (where hypsodonty explained much more variance in mandible shape). Digestive strategy is deemed to interplay with hypsodonty to produce different paths of adaptation to particular diets in ungulates.

KEY WORDS: **Feeding adaptation, geometric morphometrics, herbivore digestive strategy, mandible, ungulates.**

Natural selection is thought of as the process that fine-tunes biological structures to a given function. Yet, the power of natural selection is not unlimited. Fixed developmental programs, mechanical limitations, and even limited genetic variance and pleiotropy may severely confine the process of adaptation, and are often recognized as constraints, giving this term a negative nuance. Yet,

constraints may partly be positive in terms of fitness and can increase morphological diversity among clades (Schwenk 1995; Arthur 2001; Gould 2002; Brakefield 2006). Gould and Vrba (1982) refused to accept the statement that biological structures are shaped almost exclusively by adaptation, and restricted this term to the process of crafting morphological features to satisfy their current function (see chapter 10 in Gould 2002, for a lengthy treatment of this argument). But a given trait may have a role (i.e., it may be well-designed to serve a precise biological function, or being "aptive" in Gould's terminology) because it was secondarily co-opted for its current new function (= exaptation) or even was not selected at all (byproduct = spandrel). The work of Gould and other critics of the "adaptationist program" have led many to admit that the shape of biological structures could be envisaged as the final product of the interacting contributions of (phylogenetic) history, true adaptation, and biomechanical constraints in design (Seilacher 1970; Gould 2002). In a thorough review of the nature of evolutionary constraint, Schwenk (1995) equated "constraint" (in a broad sense) with "historical contingency." Within the latter, he pointed out that biomechanical, structural, and functional constraints are often invoked to explain patterns of limited morphospace occupation between clades. Yet, because they often are the result of natural (particularly stabilizing) selection (Gould 2002), they are not "true" constraints. Schwenk then proposed a strict-sense, process-based definition of constraints (still under the universe of historical contingency), including those developmental and genetic biases that limit the diversity of phenotypes on which natural selection may operate. These process-based constraints were demonstrated to effectively bound morphospace occupation (e.g. Beldade et al. 2002), although the existence of developmental constraints necessarily implies a positive bias in favor of alternative developmental pathways (e.g., developmental drive in Arthur [2001]) and gives room to the "creative" power of parallelism in evolution (Schwenk 1995; Gould 2002).

In practice, then, the shape variability of a biological structure within a clade is not necessarily due to natural selection (and ensuing adaptation) only, however well-designed it is.

The ungulate mandible is quite interesting in this regard because it is a complex biological structure dedicated to a precise functional role, mastication. It is characterized by a long cheek tooth row, separated by a large diastema from the canines (which are often reduced or lost). The angular region is enlarged with a consequent reduction of the coronoid process. This provides an attachment for an expanded masseter (Radinsky 1985; Janis 1995; Popowics and Herring 2006; Clauss 2008). Morphological variation in ungulate mandibles has usually been linked to adaptation to particular feeding habits (Gordon and Illius 1988; Solounias and Dawson-Saunders 1988; Solounias et al. 1988; Janis 1990; Solounias and Moelleken 1993; Janis 1995; Solounias et al. 1995; Pérez-Barberia and Gordon 1999; MacFadden 2000; Pérez-Barberia and Gordon 2001; Williams and Kay 2001; Mendoza et al. 2002; Mendoza and Palmqvist 2007). However, several recent studies argue against the evidence of adaptive significance of most mandibular traits in the light of phylogenetic affinity (Pérez-Barberia and Gordon 1999, 2001). Thus, it is unclear if the correlation between mandible shape and particular diets is just due to adaptation. An answer to this question would require a test of correlation between the mandible shape and a true adaptation, defined here as a heritable trait, which signifies a solution to a problem that environment presents and which appears simultaneously or soon after the new environmental condition (see Arnold 1994; Strömberg 2006). This true adaptation in ungulate mandibles is the relative molar crown height, measured as "Hypsodonty Index" (HI). In ungulate dentition, a hypsodont (= high crowned) molar is considered to be the principal (but by no means the only) adaptation for feeding on grasses (Feranec 2007; Janis 2008). Grazing is a derived feeding habit. In the Northern Hemisphere, grazing lineages appeared at the beginning of the Oligocene and greatly diversified during the Late Miocene, when global aridification induced the spread of grasslands in the higher latitudes (Janis 1989). The adaptation of hypsodont lineages to live in open habitats represented the major shift in the history of ungulate diets (Jernvall et al. 1996; Jernvall and Fortelius 2002; Janis 2008). Consequently, we consider that the part of total mandible shape variation that is correlated to HI has arisen from adaptation to grazing in the strict—gouldian—sense, although we emphasize that HI-related shape changes cannot capture the entire adaptation to grazing in the mandible (for instance, the enlarged masseter muscle typical of grazers is itself an adaptation to grazing influencing mandible shape). A great deal of mandible shape variation may be explained by either body size or the differences in digestive physiology between the two ungulate orders (see below). Yet, because neither of these traits can be thought of as an adaptation to grazing, whatever quota of mandible shape variation they account for is not to be considered adaptive, despite that it is most probably aptive to grazing.

In this study, we estimate how much mandible shape variation is explained by phylogeny, hypsodonty, and body size in several ungulate subfamilies. Before illustrating the statistical methods we applied, we first describe how different mandible shapes have been discussed in literature in terms of adaptation to particular feeding habits, then explore their relationship to hypsodonty and report the proposed links between mandible shape and both digestive strategy and body size.

THE LINK BETWEEN SHAPE AND FEEDING CATEGORIES

In ungulates, feeding habits are usually defined from browsers to grazers according to the degree and amount of abrasive material in food (dust, grit, and phytoliths). Pure browsers feed on soft

matter such as berries and dicot leaves (Janis 1995). Grazers feed almost exclusively on grasses, whereas mixed feeders have intermediate diets. The diet of grasses entails extensive consumption of abrasive material, both endogenous (phytoliths) and exogenous (grit, dust). Thus, grazers show distinctive patterns of increased tooth wear at both macro-scale (mesowear) and micro-scale (microwear, related to scratches along the tooth surface) (Walker et al. 1978; Solounias et al. 1988, 1994; MacFadden et al. 1999; Fortelius and Solounias 2000; Solounias and Semprebon 2002). As reported above, the solution to this intense tooth wear was the independent acquisition of hypsodont molars in several ungulate lineages (Stirton 1947; Janis and Fortelius 1988). Some large herbivores, such as many South American notoungulates, North American taeniodonts, and the giant Asian rhino *Elasmotherium sibiricum* even became hypselodont (i.e. they had ever-growing molars, as do many rodents) (Janis 2008).

The HI is defined as the ratio of molar crown height to its width at the base (Van Valen 1960). HI is distinctively greater in grazers than in browsers or mixed feeders (Janis 1995; Mendoza and Palmqvist 2007). Analyses carried out on the isotopic composition of enamel indicated that hypsodont taxa have effectively expanded their feeding niches adding grasses to their diet (Feranec 2007).

A number of morphological traits in the mandible are deemed to be correlated with increased HI, including a deeper mandible (Janis 1995; Mendoza and Palmqvist 2007), a wider attachment area for the enlarged masseter muscle (Solounias et al. 1995), a longer coronoid process (Pérez-Barberia and Gordon 1999), and a wider and flatter incisor arcade, which is narrow and prognated in browsers (Gordon and Illius 1988; Janis and Ehrhardt 1988; Solounias and Moelleken 1993; Pérez-Barberia and Gordon 2001). In addition, the ratio of the premolar to molar row lengths was shown to be low in browsing and high in grazing perissodactyls and hyracoids, but lower in grazing selenodont artiodactyls compared to browsing artiodactyls (Janis 1995; Greaves 2008).

Almost any piece of the mandible has been described in terms of adaptation to a particular feeding habit. As a matter of fact, mandible shape is used to derive feeding habits in extinct species (Solounias et al. 1988; Solounias and Moelleken 1993; Janis 1995; Spencer 1997; MacFadden et al. 1999; MacFadden 2000; Mendoza et al. 2002; Schubert et al. 2006; Rivals et al. 2008). Most of these studies implicitly interpret the correlation between morphology and function as driven exclusively by adaptation, without controlling for phylogenetic effects. Pérez-Barberia and Gordon (1999, 2001) tested the correlation of all morphologic variables we discussed above in relation to the diet of ungulates, and found out that only the length of the coronoid process, body size, and HI correlated well to dietary habits after phylogenetic inheritance was controlled for. These counterintuitive results make it questionable how much adaptation to diet on grasses (as embodied in increased hypsodonty) has actually reshaped the mandible to exploit grasslands.

THE LINK BETWEEN SIZE AND FEEDING CATEGORIES: THE IMPORTANCE OF DIGESTIVE STRATEGY

The significant effects of body size on diet are probably influenced by digestive strategy. Ruminants are fore-gut fermenters. Their complex anterior digestive tract is designed to provide the metabolism of cellulose (the main component of plant cell walls) via the activity of bacteria and other microorganisms living in the rumen. Conversely, in hindgut fermenters such as perissodactyls, the digestion of cellulose occurs in the enlarged caecum and colon. The ruminant system has long been deemed inherently superior to hindgut fermentation (see discussion in Janis 1976) because it is better at extracting nutrients from the food. A ruminant can subsist on lower absolute food quantities than a hindgut fermenter of the same size. Yet, the fore-gut fermentation system most probably presents size-dependent disadvantages because longer food retention time in small-sized ruminants limits their ability to cope with the fast metabolism their size requires (smaller mammals such as rodents and lagomorphs are mostly hindgut fermenters). At large body sizes, the great absolute food quantity required makes ruminant's food retention time a limiting factor as well, and larger ungulates (such as rhinos and proboscideans) are hindgut fermenters. Demment and Van Soest (1985) calculated a size limit of 600–1200 kg for effective fore-gut fermentation (see Clauss and Hummel [2005] for a comparable estimation). Clauss et al. (2003) demonstrated that the upper size limit is lower for grazing than for browsing ruminants because grass, as a tough food, has longer passage time in the ruminoreticulum, and an expanded fore-gut (a viable solution for the long retention time problem) reduces water absorption in the colon, to the extent that only the semiaquatic hippopotamus (which, additionally, has a slow metabolism) can subsist on a diet of grasses in spite of being very large.

Digestive strategy affects mandible shape as well. Janis and Constable (1993, see also Janis 1995) suggested that the long premolar row in equids is a direct consequence of it because hindgut fermenting grazers chew their food more than grazing ruminants (see Janis and Fortelius [1988] for a similar contention). Thus, the premolar to molar row lengths ratio, which is useful to distinguish grazer from browsers within ungulate orders (see above) might have been influenced by their different digestive strategies.

SHAPE IN LIGHT OF PHYLOGENY AND ADAPTATION TO THE GRAZING HABIT

Each biological structure has a shape, and shape must be read in terms of the correlation of its variation to phylogeny, adaptation,

and their interplay. We used geometric morphometrics to model ungulate lower jaw shape and studied its variation in the light of phylogenetic affinity by using five alternative tree topologies. First, we estimated the correlation of HI and mandible size to the shape of different mandibular parts to see if they covary. A concerted variation would suggest that the influence of HI (a true adaptation to grazing) or mandible size, either, map on different regions of the mandible simultaneously. Then, we performed a regression analysis of both mandible size and HI on mandible shape. In a second round of regressions, we took phylogenetic effects into account by applying phylogenetic generalized least squares (PGLS, Martin and Hansen 1997) and variation partitioning (Desdevises et al. 2003).

The PGLS analysis fits the regression of a given independent (X) variable on a given dependent (Y) variable via the GLS procedure, by using a specific hypothesis on the distribution of residuals around Y. This hypothetical distribution of residuals is drawn from a phylogenetic tree (Rohlf 2001, 2006c; Adams 2008, and see below for details).

Variation partitioning computes multiple multivariate regressions of a number of independent variables, including "phylogeny," on a set of dependent variables. For both PGLS and variation partitioning, we used the shape variables (represented by a matrix of shape vectors obtained from geometric morphometrics) as the dependent variables. The independent variables were the mandible size (a proxy for body size), hyposodonty index, and (only in the case of variation partitioning) phylogeny.

To take into account the effects of different digestive strategies, we repeated variation partitioning on nested subsamples of Artiodactyla and Perissodactyla. All perissodactyls are hindgut fermenters and all artiodactyls are fore-gut fermenters. Thus, if digestive strategy is of any influence on mandible shape either directly (as with the ratio of premolar to molar row lengths) or indirectly via the allometric effects of body size on shape, we expect the intensity of phylogenetic effects to decrease in the "nested" analyses.

Materials and Methods

SAMPLE SIZE

We examined 107 mandibles belonging to 80 species of both extant and extinct ungulates from the Paleocene to Recent (Appendix S1). The number of specimens per species varies between one (most extinct species) to four (Appendix S2). Where available, we included at least two adult specimens with fully erupted dentition, not considering sexual dimorphism (Appendix S2). The species included belong to the families of Camelidae, Bachitheridae, Giraffidae, Bovidae, Moschidae, Palaeomerycidae, Hoplitomerycidae, Cervidae, Rhinocerotidae, Tapiridae, Equidae, and Palaeotheridae for a total of 22 subfamilies (Appendix S1). Our sampling effort was aimed at covering as much as possible the historical morphological variability (in mandible shape) in each clade and subclade. For this reason, we focused on fossils and homogenized the sample in extant forms accordingly. This inevitably adds an error due to the poor representation of intraspecific variability (Cardini and Elton 2007). However, as all of our analyses were performed at the interspecific level, we tested the importance of intraspecific variability via a randomization test (Appendix S3), which supports the assumption that intraspecific shape variation is negligible as compared to the interspecific. We similarly checked the importance of measurement error (Appendix S3).

As preliminary analyses, we validated the assumptions, commonly described in literature, that (1) mandible shape is significantly correlated to feeding categories, (2) HI is a good proxy for grazing, and (3) HI is correlated to mandible shape. Because diet data are only available for extant species, we restricted these latter tests to extant species (Appendix S3).

DATA ACQUISITION

All mandibles were photographed in a lateral view with a Nikon 995 digital camera (Nikon Inc., Japan) at 2-m distance with procedure described in Zelditch et al. (2004, pp. 39–46). The 2-m distance reduces distortion due to the camera lens (cf. Raia 2004; Meloro et al. 2008) and the mandible positioning was standardized to minimize photographic error among specimens (Mullin and Taylor 2002; Cardini and Tongiorgi 2003; Zelditch et al. 2004).

The software tpsDig version 2.09 (Rohlf 2006a) was used to digitize eight anatomical landmarks on each digital image as representative of the overall mandibular form (shape + size) in ungulates. The landmarks record premolar and molar row relative lengths, mandible depth both below the tooth row and in its posterior part, and the position of the condyle. These parameters are easily recognized in both extant and extinct taxa and they have been usually included in previous studies on mandibular morphology (cf. Janis 1990, 1995; Mendoza et al. 2002, Fig. 1). Landmarks 1 and 2 were placed at the beginning and at the end of the premolar row (Fig. 1). Landmark 3 was placed at the rear margin of m3 (lower third molar). These landmarks were placed at alveolar edges instead of directly on the teeth to allow the inclusion of mandible specimens where teeth were shed. Landmarks 6–8 represent, respectively, the projections onto the ventral edge of the corpus of landmarks 1–3 perpendicular to the chord uniting landmarks 1 and 3 (Fig. 1). Landmark 4 was located at the highest point of the condyle. Landmark 5 was located at the intersection of the ventral edge of the corpus, drawn from the landmark 3 at an angle of 45° with the line perpendicular to the line between the landmarks 1–3. Landmark 5 is quite informative about the dimension of the ascending ramus of the mandible, which was suggested to be important in distinguishing between browsers and grazers

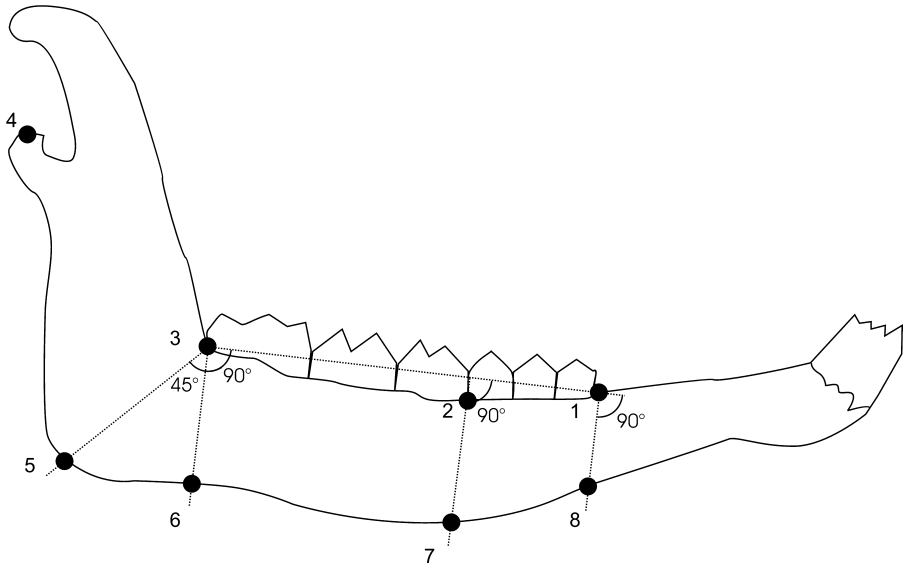

Figure 1. Diagram of an ungulate mandible showing the position of the eight landmarks chosen for shape analyses.

(Janis 1990, 1995; Mendoza et al. 2002). No landmark was placed at the position of the coronoid process because of the scarcity of fossil specimens with intact coronoids.

Landmarks 1–4 are type "2"; which are anatomically homologous according to Bookstein's terminology, whereas landmarks 5– 8 are type "3" which are geometrically homologous (Bookstein 1991).

Although sliding landmarks or shape outline methods can give a better representation of overall mandible shape, we preferred not to use them because most fossil mandibles are not complete especially in the posterior corpus edge. Furthermore, sliding landmarks are less than appropriate descriptors for our sample size because they would increase the number of shape variables in statistical analyses, hence requiring a larger sample size.

SIZE AND SHAPE DATA

After digitalization, we applied geometric morphometrics (gmm) to extract both size and shape data from two-dimensional (2D) coordinates of landmark configurations. The landmark coordinates were superimposed applying the generalized Procrustes analysis (GPA) (Rohlf and Slice 1990; Dryden and Mardia 1998). This procedure minimizes the sum of squared distances between all landmark configurations and the mean shape (called "consensus" configuration) after scaling the size of each configuration to unity. Size is here quantified as centroid size (CS) that is the square root of the mean squared distance from each landmark to the centroid of the landmark configuration (sensu Bookstein 1989).

When more than one specimen was available for a given species, we calculated the consensus shape via GPA and used its 2D coordinates in all successive (interspecific) analyses. When only one individual was available (most fossils), we assumed it represented the nominal species shape (see Appendix S3 for more explanations).

Shape data were extracted after the Procrustes registration as affine (Uniform) and nonaffine (Partial Warps) components of the bending energy matrix. This matrix describes the energy necessary to bend an infinitely thin metal plate, on which the landmark configurations are assumed to be printed. Regional deformations of the plate occur to fit one landmark configuration to another, and they are graphically represented as deformation grids under thin plate spline (TPS, Bookstein 1989) visualization.

Shape distances among landmark configurations were quantified by the Procrustes distances, which define Kendall's shape space (Dryden and Mardia 1998; Rohlf 2000a,b) that is non-Euclidean. Shape data were obtained from an Euclidean space that is actually tangent in the consensus to the Kendall's space. (Dryden and Mardia 1998; Rohlf 2000a,b). The software tpsSmall version 1.20 (Rohlf 2003) was used to verify that distortion introduced by this projection is insignificant. Principal components analysis (PCA) was performed on partial warp scores and uniform components, using the tps suite software (Relative Warps version 1.44; Rohlf 2006b), to explore the major shape variation among the taxa considered. Principal component axes (named relative warps, RW, in the gmm literature) were then extrapolated setting the distortion parameter alpha to zero (Rohlf 1993), which results in equal scaling of each regional shape variation. Raw shape data (PWs and Uniform components) were used in subsequent statistical analyses to test for the relative contribution of size or hypsodonty on mandible shape variability.

SIZE AND HYPSODONTY

Size may represent an important source of variation in the shape of biological structures (Bookstein 1989). In this regard, gmm

allows testing for such effect by CS, which represents the size of the analyzed landmark configurations. Theoretically, it is expected that CS of the mandible configuration is correlated to body size in ungulates. We verified this hypothesis by testing the relationship between CS and body mass in extant ungulates included in this study, using a nonparametric Spearman correlation test. For all the statistical analyses, CS has been transformed to natural logarithms (Dryden and Mardia 1998) as have the species mean body mass values (expressed in ln grams).

When multiple specimens were available for a given taxon, we took the average CS as representative of each species. In the other cases, the CS value of a single specimen was assumed to be the representative estimation of species mean size (cf. Cardini and Elton 2007). Mean body mass values were obtained from literature (Smith et al. 2003) because they were not directly available from the museum specimens.

Multivariate regression was employed to test the effect of both ln CS and HI on mandible shape. In this test, affine and nonaffine components of shape are the dependent variables, whereas ln CS or HI are the independent ones (Monteiro 1999; Zelditch et al. 2004). The software tpsRegr 1.34 (Rohlf 2007) was used to test the significance of regressions. Hypsodonty data were obtained from literature or directly from museum specimens (on a number of specimens for each species and then averaged). In a minority of cases, when no unworn teeth were available, we had to rely on measurements performed on sister taxa (Appendix S2). Residuals of the regression of HI on shape showed nonlinear relationships with the predicted values for a number of PWs (i.e., the dependent variable). Hence, HIs were transformed to ensure linearity. To sort among different transforms (log, ln, sin, tan, and rank) we selected that one minimizing Cook's distances of the residuals. Cook's distance is a measure of the influence of single observations on the regression coefficients (Hair et al. 1998). The rank transform provides the best reduction in Cook's distances for dependent variables showing nonlinear residuals plot, and was consequently selected. We emphasize, though, that using logged HIs gave quantitatively very similar results in all tests (not shown).

CORRELATION OF MANDIBLE PARTS TO HI AND CS

To test the correlation between different mandible parts and HI, we partitioned the mandible into three "portions" by using interlandmark (linear) distances calculated with the software Tmorphgen6 (Sheets 2006). A first "portion" was approximated by summing interlandmark distances 3–4 and 4–5. It is a proxy for the "size" of the ascending ramus. It is expected to grow large in grazing species. The second "portion" captures mandible depth below the molar row. It is represented by the sum of interlandmark distances 3–6, 2–7, and 1–8. Again, this is expected to correlate strongly with HI because it is influenced directly by crown height for purely constructional requirements. The last "portion" is indeed the premolar to molar row lengths ratio (ratio of interlandmark distances 1–2/2–3). It is expected to be related to diet but inversely between artiodactyls and perissodactyls (Janis 1995). These latter correlations, if verified, are deemed to depend on the different gut anatomies of the two orders and not on HI. For this assumption to be true, the correlation between HI and premolar to molar row lengths ratio must be significant within orders but not in the entire (pooled) sample.

TESTS FOR PHYLOGENETIC INHERITANCE

Even though hypotheses on the evolution of biological structures could be tested using both parametric and nonparametric statistical methods, it is important to note that species biological data are usually not independent (Felsenstein 1985; Garland et al. 2005). In fact, most interspecific data analyses assume that biological traits are not inherited from one ancestral species to its descendant. However, that is obviously not the case in the majority of both behavioral and morphological traits (Blomberg et al. 2003).

Comparative methods allow taking into account trait variability due to shared ancestry in interspecific data (Garland et al. 1992), hence validating biological traits correlation. A number of methodologies have been proposed (Miles and Dunham 1993; Garland et al. 2005; Adams 2008) and all of them incorporate species' traits interdependence via a phylogenetic tree. For studies concerning fossil species robust phylogenies at the species level are usually unavailable. To overcome this problem, we used trees resolved down to the subfamily level (subfamilies are then polytomies, see Finarelli and Flynn 2006; Meloro et al. 2008).

We used five alternative ungulate phylogenies in PGLS multivariate regressions of both ln CS (independent variable) versus shape (dependent), and HI (independent) versus shape (dependent) (see Appendix S4 for details and references about the five phylogenies, and for the five tree topologies). PGLS takes phylogeny into account by translating the tree topology and branch lengths in a specific hypothesis about the variance–covariance matrix of the error term ε, in the regression equation $Y = \beta X + \varepsilon$, under a Brownian motion model of evolution (Rohlf 2001, 2006c; Blomberg et al. 2003; Adams 2008; Lavin et al. 2008). The initial regression equation is then transformed via GLS procedure in $Y' = \beta X' + \varepsilon'$, which has uncorrelated errors with equal variance (Rohlf 2001, 2006c). In multivariate regression, a potential problem with the interpretation of PGLS results (and with any other comparative method specifying the variance–covariance matrix of residuals) is that Y is related to both ε and X, but phylogenetic effects are included only in ε. Thus, values of Y may show phylogenetic resemblance if values of X do, even when values of ε are independent (Lavin et al. 2008). In the biological context explored here, this means phylogenetic resemblance (= phylogenetic signal, Blomberg et al. 2003) in Y (shape variables) may

remain after PGLS regression of X (ln CS or HI either) on Y, if the X variable shows strong phylogenetic signal. To explore this possibility, we calculated the K statistic (Blomberg et al. 2003) for both HI and ln CS, by using the software PHYSIG.M kindly provided by T. Garland.

For each species, branch lengths were calculated as the difference in million years between its first appearance (FA) in the fossil record and the estimated age of the subfamily to which it belongs (Finarelli and Flynn 2006). Species FAs and subfamilies ages were taken either from literature or online databases (NOW, http://www.helsinki.fi/science/now/; and the Paleobiology Database, http://paleodb.org/cgibin/bridge.pl) (Appendix S2). Covariance matrices were extracted from each phylogenetic topology by using the module PhyloCov in NTSYS 2.2n (Rohlf 2006d). PGLS was performed with the same software.

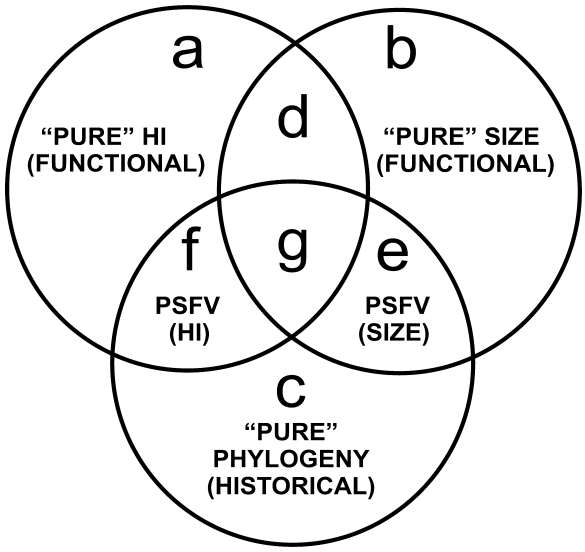

Figure 2. Schematic depiction of the three factors analyzed in partition variation meant to illustrate both their individual contribution to shape variance (i.e., fractions *a*, *b*, and *c*) and their interacting components. The interaction of a functional factor with phylogeny gives its phylogenetically structured functional variation (PSFV, i.e., fractions *d* and *f*, see the text for further explanation).

VARIATION PARTITIONING

A number of phylogenetic comparative methods have been proposed in literature. Although most of them perform quite well in a variety of experimental conditions (Martins et al. 2002; Garland et al. 2005), each method has its own assumptions and limitations (Rohlf 2001; Freckleton et al. 2002; Martins et al. 2002; Lavin et al. 2008). As a consequence, it is often suggested to use more than one comparative method on the same set of data to test the same hypothesis (Martins et al. 2002; Garland et al. 2005). Therefore, in addition to PGLS, in this study we also used variation partitioning (Desdevises et al. 2003). This method divides the total variation of a dependent variable into two or more sets of explanatory variables in a phylogenetic context. It is an extension of the partitioning method used in ecological studies (Borcard et al. 1992; Borcard and Legendre 1994; Legendre and Legendre 1998; Diniz-Filho and Bini 2008).

The basic procedures in variation partitioning involve a number of linear regressions followed by subtractions. We first performed seven linear regressions of the dependent variable (shape, as represented by PWs and uniform components vectors) on phylogeny, HI, ln CS, and their multiple combinations (e.g., phylogeny and HI). Each of these regressions produced a portion of the total variation in Y, explained by the independent variable (indicated by the R^2 of the regression). We had three independent variables, each including four fractions (e.g., HI includes fractions a, d, f, g, Fig. 2). Three of these four fractions were shared with other independent variables. The fourth fraction (a, b, and c; Fig. 2) represented a "pure" contribution of the independent variable to the dependent variable's total variance. Hence, subtractions (of R^2 values) can be used to quantify the fractions a to g (Fig. 2), and to calculate unexplained variance in the dependent variable. All the fractions except d, e, f, and g can be tested for significance via partial regressions. In sum, this method allows an assessment of the total variation of a dependent variable: (1) as explained exclusively by phylogeny; (2) as explained exclusively by functional variables; or (3) as explained by the overlap (interaction) of these variables in what is known as phylogenetically structured functional variation, PSFV (Westoby et al. 1995; Cubo et al. 2005, 2008, see Fig. 2).

A major difference between variation partitioning and PGLS is that the former quantifies the variable "phylogeny." In keeping with Diniz-FIlho et al. (1998), phylogeny was translated in a phylogenetic distance matrix with the Stratigraphic Tools module for Mesquite (Josse et al. 2006). Then, principal coordinate scores were extracted from this matrix.

Rohlf (2001) criticized matrices based on path length distances of the type used here stating that they do not represent the expected amount of independent evolution since divergence from a common ancestor, although ultrametric and path length distances are *usually highly correlated* (Rohlf's words in italics). We do not believe that the height above the tree root of the common ancestor is the most appropriate metric here because species in a pair may have very unequal duration (differing, in some instances, by more than 40 million years). Thus, by using covariance between species pairs we would ignore that these two species could have had very different periods of time to evolve their shapes. We, nonetheless, preliminarily checked the correlation between distance matrix and variance–covariance matrix for each phylogeny in this study, using Mantel's test. We always found very high and strongly significant correlations (not shown).

Diniz-Filho et al. (1998) proposed to use only the principal coordinates that were representative of phylogeny, by applying the broken-stick model (Frontier 1976). Rohlf (2001) and Martins et al. (2002) noted that this approach does not allow taking the entire phylogeny into account. Desdevises et al. (2003) similarly criticized the broken-stick criterion and alternatively proposed to test principal coordinates individually to see their influence on the dependent variables. In keeping with this criticism, we retained all the principal coordinates explaining a significant fraction of PWs + uniform components matrix variation up to the 95% in cumulative variance explained. Variation partitioning was computed by using the library *vegan* (Oksanen et al. 2008) for R (R Development Core Team 2008).

Results

CORRELATION OF MANDIBLE PARTS WITH HI AND CS

The correlation between HI and the size of ramus and mandible depth is always significant in the entire sample and in orders taken separately (Table 1). This suggests that HI influences (either directly or not) even mandible parts that are not directly affected by increase in molar crown height. The influence of CS on the size of ramus and mandible depth is always significant, except for the mandible depth in perissodactyls. Its influence on the premolar to molar row lengths ratio is marginally significant in the entire sample and is no longer significant when the orders are separated (Table 1).

The premolar to molar row lengths ratio does not correlate with HI when tested in all ungulates (Table 1). Significant correlations occur when the two orders are tested separately, but these significant relationships have the opposite trends (Table 1). These patterns are consistent with previous studies that suggest the relationship between digestive strategy and HI depends on taxonomic affiliation.

REGRESSION OF HI AND ln CS ON SHAPE VARIABLES

Mandible shape variation

After GPA procedure, 12 shape variables were extracted and then reduced through RW analysis. The first five RWs explain ca 95% of shape variability. RW1 accounts for 44.66% of shape variance, RW2 accounts for 28.33% of shape variance, and RW3 accounts for 10.48% of shape variance. The first two RWs are informative from a phylogenetic and (partly) on the ecologic perspective (Figs. 3A,B). On the first RW (RW1), mandible length is positively associated with the elongation of the tooth row and the anterior projection of the condyle. On RW2, the mandible deepens and molar row shortens (in proportion) as it moves from Artiodacyla to Perissodactyla (Fig. 3A). However, there is some separation of grazers from browsers along RW1 as well (Fig. 3B). Grazing perissodactyls occur mostly in the negative-values domain of both RW1 and RW2. Accordingly, this quadrant contains grazing equids, woolly rhino, *Elasmotherium* and *Ceratotherium* as well as strictly grazing artiodactyls, bovids *Bos* and *Bubalus*. Mixed feeders and browsers occur in the positive values domain of RW2. Their mandibles are characterized by an elongated and slender corpus (Fig. 3B). Most extinct taxa overlap with extant species although peculiar shapes occur among the extinct group (e.g., the primitive equid *H. leporinum*).

Regression of HI and ln CS on shape variables

The correlation between ln CS and body size in extant species is highly significant ($N = 43$, $R^2 = 0.606$, $P < 0.001$). This means that the size of our mandible configuration is in general a good descriptor of species body size.

Multivariate regression indicates that a significant relationship occurs between ln CS and shape data (PWs + Uni) (Wilks' lambda = 0.507, $F_S = 5.426$; df = 12, 67.0; $P < 0.001$). This observation is supported also by the Goodall F test

Table 1. Correlations between HI, ln CS, and "size" of mandible portions. Significant correlations are in bold face.

		n	Ln CS		HI	
			r	*P*	*r*	*P*
Entire sample		80				
	p/m ratio		0.235	0.036	0.036	0.752
	"Size" of the ramus		**0.782**	0.000	**0.484**	0.000
	Mandible depth		**0.630**	0.000	**0.400**	0.000
Artiodactyla		50				
	p/m ratio		0.053	0.717	**−0.505**	0.000
	"Size" of the ramus		**0.687**	0.000	**0.651**	0.000
	Mandible depth		**0.527**	0.000	**0.448**	0.001
Perissodactyla		30				
	p/m ratio		−0.065	0.734	**0.512**	0.004
	"Size" of the ramus		**0.700**	0.000	**0.496**	0.005
	Mandible depth		0.203	0.282	**0.666**	0.000

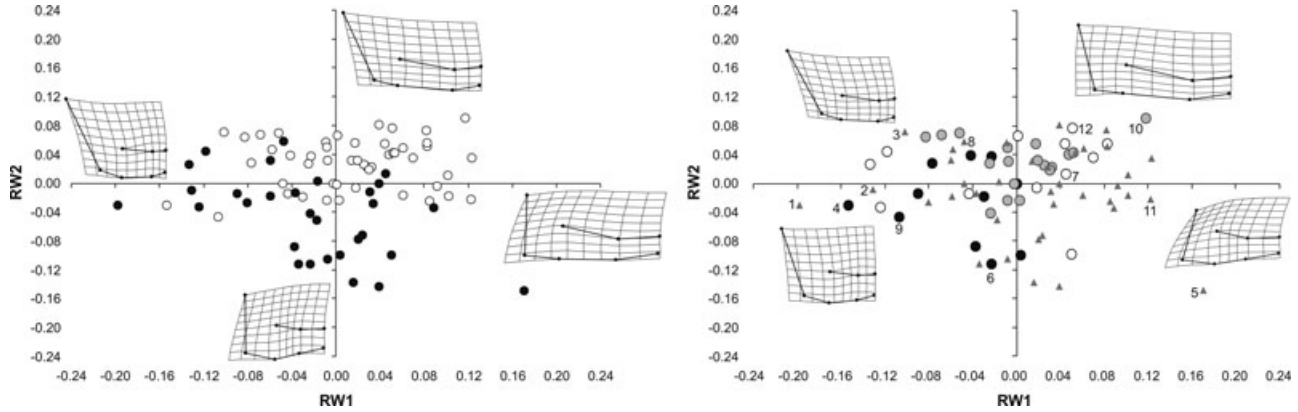

Figure 3. **Scatter plots of the first versus the second relative warp. On the left (A) all species are plotted, perissodactyls (solid circles), and artiodactyls (open circles). The plot (B) on the right shows extant browser (black circles), mixed feeders (gray circles) and grazers (white circles). Extinct forms are in gray triangles. Grazer labeled species are: extinct woolly and Asian giant rhinos (*Coelodonta antiquitatis* 1 and *Elasmotherium sibiricum* 2); the auroch (*Bos primigenius* 3), the water buffalo (*Bubalus murrensis* 4). The labels for *Hyracotherium leporinum* (5) and Burchell's zebra (*Equus burchelli* 6) are shown to indicate the position of extinct and living horses. Among browsers the black rhino, *Diceros bicornis*, is labeled as 7 whereas the extinct *Stephanorhinus kirchbergensis*, a mixed feeder, is labeled (8). Artiodactyls are represented by the grazer argali (*Ovis ammon* 9), the browser muntjac (*Muntjacus muntjacus* 10), the extinct *Amphimoschus elegans* 11, and the browser brocket deer (*Mazama americana* 12).**

(Generalized Goodall F-test: $F = 12.798$, df $= 12, 936$; $P < 0.001$) with ln CS explaining 13.98% of shape variability. Shape deformation due to size is associated with an overall stretching of the mandible in smaller forms in which the corpus is more elongated and slender (Fig. 4). In larger taxa (e.g., rhinos), the premolar and the molar rows are shorter relative to the mandible length, the region behind the molars is larger, whereas the condyle appears to be farther from the last molar (see right extreme on Fig. 4). Mandible depth under molar teeth becomes larger as size increases, whereas the premolar row becomes relatively shorter.

A significant relationship occurs between hypsodonty index and mandible shape (Wilks' lambda $= 0.422$, $F_S = 7.647$, df $=$ 12, 67.0, $P = 1.087 \times 10^{-008}$). Goddall F test is significant as well ($F = 6.615$, df $= 12, 936$: $P < 0.0001$) with hypsodonty rank values explaining 7.75% of shape variation. In brachydont forms, the mandible is shallower with the molar row being similar in length to the premolars (see the extreme case of *Tapirus indicus*, left side of Fig. 4).

TESTS FOR PHYLOGENETIC INHERITANCE

PGLS

Regardless of the phylogenetic topology used, the relationship between ln CS and shape variables is not significant (Table 2). This means that this relationship in ungulate mandibles, although significant, is superseded by the effect of shared ancestry. The opposite is true of HI, where the relationship between hypsodonty ranks and shape is always significant (Table 3).

PHYLOGENETIC SIGNAL

As estimated by the K statistic (Blomberg et al. 2003) phylogenetic signal in HI is stronger than in ln CS. Within the two ungulate

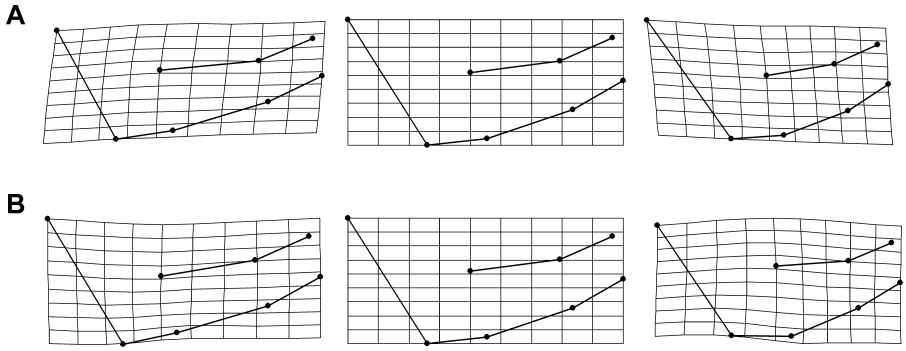

Figure 4. **(A) Shape deformation obtained regressing shape versus ln CS; at ln CS of 4.44 (*B. insigne*), of 5.39 (*R. timorensis*), and of 6.3 (*C. antiquitatis*). (B) Shape deformation occurring at the hypsodonty rank 1 *T. indicus* (HI = 0.76), 37 *T. strepticeros* (HI = 2.29), and 80 *B. bonasus* (HI = 6.12).**

Table 2. PGLS analyses performed using five different phylogenetic covariance matrices to test the association between ln CS and mandible shape.

	Wilks' lambda	F_S	df1	df2	P
Phylogeny 1	0.647	1.357	24	134	0.140
Phylogeny 2	0.639	1.399	24	134	0.119
Phylogeny 3	0.648	1.355	24	134	0.142
Phylogeny 4	0.648	1.355	24	134	0.142
Phylogeny 5	0.661	1.284	24	134	0.186

Table 3. PGLS analyses performed using five different phylogenetic covariance matrices to test the association between ranked hypsodonty values and mandible shape.

	Wilks' lambda	F_S	df1	df2	P
Phylogeny 1	0.469	2.566	24	134	3.50×10^{-04}
Phylogeny 2	0.514	2.204	24	134	0.0025
Phylogeny 3	0.471	2.550	24	134	3.82×10^{-04}
Phylogeny 4	0.471	2.548	24	134	3.86×10^{-04}
Phylogeny 5	0.471	2.555	24	134	3.73×10^{-04}

orders, this difference is stronger in artiodactyls and much weaker in perissodactyls (Table 4). For the latter group, ln CS is not significantly different from 0, irrespective of the phylogenetic topology used. The phylogeny 4 in artiodactyls is an exception because the value K in HI and ln CS is similar.

VARIATION PARTITIONING

With any of the five phylogenetic topologies, the influence of phylogeny is much higher than those of HI and size. Corresponding adjusted R^2 range from 29% to 36% (Table S1). This figure is similar when only the phylogenetic effect is used because "pure" phylogeny explains 27–35% of total shape variation, and it is always a significant contribution. Total CS contribution is significant, and ln CS explains some 12% of the total shape variation. Yet, consistently with PGLS results "pure" ln CS effect is not significant in four of five topologies, ranging from 0.4% to 12.6% of variance explained. The influence of CS becomes significant only when the PSFV due to phylogenetic effect (which explains 5–11% of total variation, depending on the phylogeny tested) is added to this pure component (Table S1).

Total HI effect is significant and explains 6.6% of the total variation, a figure very similar to that obtained by PGLS. Yet, the "pure" HI effect is much more evident, ranging from some 5 to 14% of shape variation, depending on which phylogeny is used. Interestingly, the interaction of size and HI explains 18% of total variation, consistently across all phylogenies, and this interaction is strongly significant. This means that, taken together, the effect of body size plus HI on mandible morphology is not dissimilar to the contribution of phylogeny alone.

Nested analyses on artiodactyls indicate an overwhelming importance of HI (explaining 20–26% of total variance) to mandible shape variation, which is always the most important contribution and the only one remaining significant, when "pure" components are tested, in four of five phylogenies. CS still explains a negligible and insignificant portion of shape variation, and its PSFV remains much more important than its pure component.

In odd-toed ungulates, phylogeny still remains the most important factor, explaining as much as 38% of total variation, although HI becomes much more important than in the whole-sample analyses, explaining 20% of total variation. The contribution of HI and phylogeny are always highly significant both when tested as pure components, and when interaction effects are included. PSFV in HI is positive but negligible. PSFV in ln CS still explains 6–8% of total variation, and it is much more important than ln CS alone but never exerts any significant contribution to shape variance.

Table 4. Phylogenetic signal (K) in HI and ln CS in the phylogenetic topologies used here.

	Phylogeny 1		Phylogeny 2		Phylogeny 3		Phylogeny 4		Phylogeny 5	
	K	P	K	P	K	P	K	P	K	P
All species										
HI	1.092	<0.001	1.361	<0.001	1.308	<0.001	1.492	<0.001	1.642	<0.001
ln CS	1.004	<0.001	1.043	<0.001	0.769	<0.001	0.823	<0.001	0.767	<0.001
Artiodactyls										
HI	2.003	<0.001	1.508	<0.001	1.959	<0.001	1.261	<0.001	1.958	<0.001
ln CS	0.767	0.064	0.779	0.248	0.774	0.054	0.901	0.001	0.771	0.058
Perissodactyls										
HI	0.580	0.240	0.481	0.563	1.079	<0.001	1.060	0.001	1.057	<0.001
ln CS	0.436	0.706	0.412	0.776	0.560	0.391	0.476	0.204	0.558	0.416

Discussion

Hypsodonty, phylogeny, and digestive strategy all contribute to shape variation in ungulate mandibles. Widespread, functionally guided morphological resemblance in the two major clades (Artiodactyla and Perissodactyla) is well apparent. However, phylogeny is not a process, and saying that one-third of total shape variation among ungulate species (in our analyses) is explained by phylogeny means only that closely related taxa have similar shapes. It is not even possible to say if this matters to the mandible proclivity to adapt to different feeding regimes over evolutionary times (Revell et al. 2008).

In terms of adaptation to grazing, the hypsodonty-related shape changes are causally informative about the functional design of ungulate mandibles. Taxa with hypsodont molars have wide angular processes of the dentary and their deep mandibles are posteriorly expanded, which make their mandibles quite distinctive from the slender mandibles of the browsers (Fig. 3B). These characteristic features in grazers depend on their much higher tooth crowns and, most probably, on their expanded masseter muscles (Popowics and Herring 2006; Clauss 2008).

Hypsodonty explains 7.75% to 14% of the total shape variation, depending on the inclusion of another explanatory variable, mandible size. On average the hypsodonty value is always higher than the mandible size, and it gives a significant contribution to shape variance. In PGLS analyses, we found hypsodonty to remain significantly related to shape when phylogeny is accounted for. Variation partitioning gave a similar indication and suggested that much of this effect is due to autapomorphic shape change at the subfamily level (our phylogenies are not resolved at the species level), that occurred independently among odd- and even-toed ungulates.

Hypsodonty-related shape variation involves all mandible parts. Interestingly, it affects the ratio of premolar to molar row lengths in the opposite directions in the two orders. Yet, these inverse relationships are probably guided by different digestive strategies and gut anatomies rather than hypsodonty. Greaves (1991) contended that the short premolar row in long-faced ungulates is a consequence of mandible biomechanics (a structural constraint) but Janis (1995) argued that equids are also long-faced but have long premolar rows. She stated that this different design in equids depends on their digestive physiology. Horses are hindgut fermenters, which means they need more chewing at the beginning of food processing than fore-gut fermenters, hence the longer tooth row. Thus, shape variation induced by hypsodont molars becomes the only major source of shape variation when the orders are analyzed individually (thus factoring out their differences in digestive strategy). This interplay of hypsodonty and digestive strategy leads to morphologically different mandible shapes associated with the same demand of living in grasslands. Indeed, when ungulates are split into two orders (and the effect of different digestive strategies is factored out), hypsodonty becomes the only major source of shape variation in artiodactyls (where phylogenetic effects disappear altogether) and is still very important in perissodactyls (where phylogeny continues to be the most important shape determinant nonetheless). Hypsodonty in artiodactyls shows a very strong phylogenetic signal, which means that closely related species have more similar than expected (after accounting for phylogenetic effects) HIs. Within perissodactyls, the phylogenetic signal in hypsodonty is much weaker.

Body size, whose physiological effect on diet is so profound, explains a mere 1–14% of total shape variation in the mandible, depending on the inclusion of its interaction with hypsodonty. And this influence disappears altogether when phylogeny is controlled for, both testing all species together, and artiodactyls and perissodactyls separately. Indeed, these results do not imply that size is unimportant. As a matter of fact, in ungulates size, especially in combination with hypsodonty, offers a robust indication of evolutionary shape changes (Table S1). The chances are that any species larger than one metric ton with hypsodont teeth is a grazer. However, chances are even higher that this species is a hindgut fermenter. In this study all hindgut fermenters are perissodactyls. Thus, we argue that the effect of body size appears so small just because body size itself is subjected to a strong anatomical constraint, digestive physiology, which does not vary within clades.

One could be tempted to assume that variance due to phylogeny represents a limit (constraint) to the power of adaptation as well. Yet, we argue this is probably not the case. Unfortunately, the term constraint is often misused in literature (Schwenk 1995), and confounded with phylogenetic inertia (Blomberg and Garland 2002). Orzack and Sober (2001) define phylogenetic inertia as the influence of the initial state of a trait on its final state. Hansen and Orzack (2005) define "phylogenetic constraint" as the multitude of causes underlying "phylogenetic inertia," which they state "refer to the fact that a trait may not be perfectly adapted to its current environment because of its evolutionary history." Still, Revell et al. (2008) demonstrated that a pattern of phylogenetic inertia gives no information about the underlying selective regime. In our case, there is a strong physiological (Janis 1976) and morphological (this study and Janis 1995, 2008) evidence that grazing evolved among both perissodactyls and artiodactyls along different pathways, and there is no reason to believe that any ungulate clade is maladapted to grazing. Hence, evolutionary constraint acted as a channeling force in the production of novel morphologies to be adapted to grazing. These morphologies represent a trade off between the largely plastic mandible shape and the conservative digestive strategy. Inertia, in our case, must then refer to the resemblance between closely related species that does

not fit in this trade off, and should include preservation of feeding habits along phyletic lineages to some extent. Thus, even if we found hypsodonty to explain some 10% of mandible shape variation in ungulates, this does not imply that only 10% of mandible is adapted to grazing. According to our definition of adaptation, this only means that a true adaptation to grazing, hypsodonty, accounts for about 10% of shape variation in ungulate mandibles, irrespective of phylogeny. Further adaptation to grazing is surely present in these mandibles, whose shapes, though, were channeled by different digestive strategies between the two ungulate orders. Digestive strategy, contrary to hypsodonty, acted like a constraint.

Patterns in the fossil record support this notion. Consider, for example, the classic idea that artiodactyls replaced perissodactyls by means of competition as climate became more seasonal and cooler during the late Eocene. This phenomenon has long been interpreted in terms of adaptation to the novel conditions and competitive replacement of odd-toed ungulates by ruminants (but see Janis 1976, 1989, 2008). Yet, most probably it was their digestive strategy, already in place before any increase in seasonality, and the consequent ability to subsist on lower absolute food quantities that let ruminants takeover (Janis 2008). It is important to stress here again that we do not consider differences in digestive strategy between odd- and even-toed ungulates to be an adaptation related to feed on grasses. That difference did not evolve to cope with the augmentation of grasslands or changes in the Eocene plant diversity. On the contrary, hypsodonty developed in most modern ungulate clades in association with drier and more open habitats. The case of the earliest hypsodont browsers, like notoungulates, although limited to a single ancient group, probably needs further investigation, for their hypselodont molars are apparently unrelated to a diet of grasses.

It is worth noticing that the indications we obtained here are only partially consistent with those of a companion study that we performed on large carnivores (Meloro et al. 2008). There, dietary features relevant to the feeding habits (i.e., carnassial cusp shape) also appeared to have had an influence on mandible shape. Yet, body size was highly significant in shaping carnivore mandibles. Whether this difference lies in the different energetic demands associated with feeding in carnivores (Carbone et al. 2007) is open to inquiry.

Conclusions

Life in grasslands and/or in generally more open environments appears to have been significantly associated with hypsodonty. Hypsodonty-related shape changes occur in all parts of the mandible in spite of phylogenetic effects, even those not directly related to the teeth. Indeed, with increased molar crown height the tooth row proportions significantly changed in opposite directions depending on the taxonomic affiliation of the species considered (either perissodactyl or artiodactyl). These differences were guided by the different digestive strategies in the clades, thus are not an adaptation in the strict sense. The shape variance explained by hypsodonty increased in both artiodactyls and perissodactyls from 14% to more than 30% and the phylogenetic effect in artiodactyls became insignificant, when the two orders are analyzed separately. The mandible size (represented by ln CS, correlated well to body size) was ineffective to explain a significant portion of shape variance, when phylogeny was controlled for. Yet, the interaction of mandible size and phylogeny was strong and significant, whereas the interaction of hypsodonty and phylogeny was extremely weak and never significant. Finally, we note that our results in this study are partially similar to those of a companion study, performed on large carnivores, where the key dietary features showed a pervasive influence on mandible shape, although with a highly significant influence of body size. This difference may well be in the different constraints (intended as a positive process channeling shape variation) that different digestive strategies exerts on ungulates but not on carnivores.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. List of species and references used to calculate branch lengths.
Appendix S2. List of specimens analyzed.
Appendix S3. Sampling issues and validation of assumptions.
Appendix S4. Description of the five alternative phylogenetic hypotheses used in this study.
Table S1. Results of partition variation analyses illustrated for all phylogenetic hypotheses.

Supporting Information may be found in the online version of this article.
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